I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail, in an envelope addressed to: MS Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: Oct. 7, 04 Signature: (XUQIONG WU)

Docket No.: COTH-P07-701
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Patent Application of:
Szostak et al.

Application No.: 09/876235 Confirmation No.: 6199

Filed: June 6, 2001 Art Unit: 1634

For: SELECTION OF PROTEINS USING RNA-PROTEIN FUSIONS Examiner: B. J. Forman

STATEMENT UNDER 37 CFR 1.608(b)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Enclosed with this Statement are the following documents: (1) declaration of Dr. Richard W. Roberts, an inventor of the above-identified application and (2) declaration of Dr. David Wilson, a corroborating witness. Collectively, these documents demonstrate that the invention described and claimed in claims 47-76 of the above-identified patent application was conceived and reduced to practice prior to October 17, 1996, the foreign priority date of U.S. Patent No. 6,361,943 ('943 patent). The enclosed testimony and documentary evidence constitute a prima facie showing under 37 CFR 1.608(b), and is sufficient to establish the conception and reduction to practice of the claimed invention prior to October 17, 1996.

STATUS AND SCOPE OF CLAIMS

The pending claims in this application are claims 47-76. Claims 47-62 have been copied substantially from the '943 patent for the purpose of provoking an interference with that patent. The correspondence of the claims of the present application and the claims of the '943 patent is as follows:

Applicants' Claim	'943 Patent Claim
47	1
48	2
49	3
50	4
51	5
52	6
53	10
54	11
55	12
56	15
57	16
58	19
59	20
60	21
61	22
62	23

Present claim 47 is directed to a molecule comprising a nucleic acid bound to a protein with puromycin, or a puromycin analog, specifically 3'-N-aminoacylkpuromycin aminonucleoside and 3'-N-aminoacyladenosine aminonucleoside. The protein portion of the

molecule is encoded by the respective nucleic acid portion. Claims 48-55 are also composition of matter claims dependent on claim 47.

Claims 56-58 are method of preparation claims dependent on claim 47. According to the methods of claims 56-58, a DNA construct containing a gene lacking a termination codon is prepared, and the DNA is transcribed into RNA. A chimeric spacer is bound to the 3'-terminal end of the RNA, followed by the bonding of a nucleoside or an analog thereof. Protein syntheses is performed to bond the gene to a translation product of the gene. In claim 56, the chimeric spacer is DNA and RNA. In claims 57 and 58 the chimeric spacer is DNA and polyethylene glycol

Claims 59, 61 and 62 are directed to a method for protein evolution simulation by selecting a molecule prepared according to the methods of claims 56 or 57, introducing a mutation into the gene portion of the molecule, and amplifying the gene portion of the molecule.

Finally, claim 60 is directed to a method for assaying protein intermolecular interaction by selecting a molecule prepared according to claims 56 or 57, and examining the intermolecular action of the protein with another protein or a nucleic acid.

A proper showing under 37 CFR 1.608(b) requires that applicants establish a prima facie case that they are entitled to judgment relative to the patentee. This criteria is satisfied when appellants establish that the invention has been conceived and reduced to practice prior to the earliest priority date of the interfering patent. i.e. October 17, 2996. MPEP 2308.02. Evidence of prior conception and reduction to practice is discussed below.

CONCEPTION

As shown by the enclosed declarations, the subject matter of present claim 47 was jointly conceived by Dr. Richard W. Roberts and Dr. Jack W. Szostak at least as early as February 10, 1994. See Roberts, ¶'s 4 and 18. The Annual Report referenced in paragraph 4 of the Roberts declaration describes a protein-nucleic acid fusion product with puromycin attached to the 3' end of the molecule. The protein is encoded by the nucleic acid. The preparation and assembly of the component parts of the fusion molecule is also described. The Annual Report describes a utility for these molecules, such as the selection of proteins with a biological function from a protein library.

Conception of the invention is also established by the published NIH grant application dated November 17, 1994. See Roberts, ¶'s 4 and 5. Since the NIH grant is an official government document, applicants submit that it is self-authenticating, and does not require further corroboration. However, corroboration is provided by Wilson (Wilson, ¶ 3).

REDUCTION TO PRACTICE

The subject matter of claim 47 has been successfully reduced to practice as shown in the Roberts declaration, corroborated by the declaration of Wilson. The Roberts declaration includes copies of laboratory notebook pages appended as exhibits. The notebook pages, as testified to by Roberts, provide substantiation for the successful preparation of molecules falling within the scope of claim 47.

The molecules of claim 47 encompass a nucleic acid bound to a protein with puromycin, with the protein being encoded by the nucleic acid. This molecule was reduced to practice by assembling its components entities as described in the Roberts declaration.

As shown in the enclosed declarations, puromycin was reacted with trifluoroacetic anhydride to block reactive groups during further reaction steps (Roberts, ¶ 11). The blocked puromycin was succinylated for cross-linking to a solid support (Roberts, ¶ 14). The product of this step, CPG-puromycin, was synthesized and used in an automated DNA synthesizer to prepare 3'-puromycin tethered oligonucleotides (Roberts, ¶'s 9, 10 and 15). The CPG-puromycin was used by Roberts to prepare RNA-puromycin conjugates (Roberts, ¶'s 16 and 17). These conjugates were then translated in vitro to prepare protein-nucleic acid fusion products of the type claimed in present claim 47 (Roberts, ¶ 19). The experimental work described herein is corroborated by Wilson (Wilson, ¶ 3).

CONCLUSION

The enclosed declarations and supporting documents establish that the invention defined by claim 47 of the above-identified application was successfully conceived and reduced to practice by Roberts and Szostak prior to October 17, 1996, the earliest priority date of the '943 patent. Accordingly, an interference should now be declared between the present application and

the '943 patent on the basis the count proposed above. Applicants request that this paper should be kept separate from the prosecution file of the application, and made available to the patentee only after an interference has been declared, and motions, if any, are filed and decided.

Dated: October 7, 2004

Respectfully submitted,

By_____

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Application No.: 09/876235

Docket No.: COTH-P07-701

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Szostak et al.

Application No.: 09/876235 Confirmation No.: 6199

Filed: June 6, 2001 Art Unit: 1634

For: SELECTION OF PROTEINS USING RNA-PROTEIN FUSIONS Examiner: B. J. Forman

DECLARATION OF DR. DAVID WILSON

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Dr. David Wilson, declare and state as follows:

1. I was a postdoctoral fellow in Dr. Jack W. Szostak's laboratory ("the Szostak Lab") at the Massachusetts General Hospital ("MGH") from December 1, 1995 to April 2000. I am presently a resident at 2462 Wyandotte Strect, Mountain View, CA 94043.

2. I have personally known both Dr. Richard W. Roberts and Dr. Jack W. Szostak, the named inventors for the above-referenced patent application, since December 1, 1995, when I first joined the Szostak Lab. When I arrived at MGH, Dr. Roberts had been working with Dr. Szostak's supervision and as a postdoctoral fellow in the Szostak Lab for more than two years and had been awarded an National Research Service Award ("NRSA") by the National Institutes of Health, usually known as an NIH Postdoctoral Fellowship.

3. When I arrived at MGH in December 1995, Dr. Roberts and Dr. Szostak had been working for more than two years on developing a system for selecting proteins and their encoding nucleic acids from a library by using RNA-protein fusion molecules, where the protein

portion is encoded by the RNA portion. By the time of my arrival, Dr. Roberts had already made an RNA-protein fusion molecule, in which the protein portion was a c-myc epitope and the RNA portion encoded the c-myc epitope. He had also been able to show the interaction of the RNA-protein fusion molecule and anti-c-myc antibody, confirming the successful preparation of the fusion molecule.

4. From December 1995 to August 1997 before Dr. Roberts finished his postdoctoral fellowship at the MGH, I had personal interactions with Dr. Roberts on a daily basis, and I had frequent personal interactions with Dr. Szostak. In addition, the Szostak Lab held weekly lab meetings at which Dr. Roberts made regular presentations of his work. Through these interactions, I became familiar with the experiments that Dr. Roberts was conducting.

The undersigned declares further that all statements made herein of his own knowledge are true and all statements made on information and beliefs are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issuing thereon.

David Wilson, Ph.D.

Date: Oct 4, 2004

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Szostak et al.

Application No.: 09/876235 Confirmation No.: 6199

Filed: June 6, 2001 Art Unit: 1634

For: SELECTION OF PROTEINS USING RNA-PROTEIN FUSIONS Examiner: B. J. Forman

DECLARATION OF DR. RICHARD W. ROBERTS

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Dr. Richard W. Roberts, declare and state as follows:

1. I, along with Dr. Jack W. Szostak, am named as an inventor of the above-identified patent application ("the '235 application") and of the subject matter described and claimed therein. Currently, I am an Assistant Professor of Chemistry in the Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125. I have held this position since 1997. Previously, I was a postdoctoral fellow in Dr. Szostak's laboratory at the Massachusetts General Hospital, Harvard Medical School from April 1993 to August 1997.

2. I have been asked to review the current status and most recent Office Action of the '235 patent application. I understand that certain claims of the '235 application are identical to those of U.S. Patent No. 6,361,943 ("the '943 patent"), assigned to Mitsubishi Chemical Corporation. I also

understand that the Examiner is requiring the applicants in the '235 application to provide a showing under 37 C.F.R. 1.608(b) to establish a prima facie case for invention of the claimed subject matter by the co-inventors prior to October 17, 1996, the earliest Japanese priority date to which the '943 patent may be entitled.

3. The invention claimed in claim 1 of the '943 patent and in the present application is a protein-nucleic acid fusion molecule, wherein the nucleic acid portion and the protein portion are covalently bonded through a substance having a chemical structure of puromycin, 3'-N-aminoacylpuromycin aminonucleoside, and 3'-N-aminoacyladenosine aminonucleoside, and wherein the protein portion is encoded by the nucleic acid portion. See Exhibit A. As evidenced *inter alia* by this declaration and the exhibits attached hereto, I and the other co-inventor, Dr. Jack W. Szostak, had conceived of and reduced to practice the claimed invention prior to October 17, 1996.

CONCEPTION OF THE INVENTION:

4. Dr. Szostak and I conceived of the claimed protein-nucleic acid fusions well before the October 17, 1996, the alleged priority date of the '943 patent. On February 10, 1994, I completed an "Annual Report" on the progress of my post-doctoral research. See Exhibit B. This Annual Report describes our goal of producing a protein-nucleic acid fusion by performing in vitro translation of an mRNA molecule having puromycin at the 3' end. As stated in the Annual Report, we undertook this research in order to develop a system for selecting proteins with a desired function from a pool of randomized proteins (a protein "library"), along with the encoding nucleic acid. The Annual Report illustrates the series of experiments that we planned for preparing the claimed protein-nucleic acid fusions: (1) preparation of puromycin in a form that is suitable for use with standard DNA/RNA synthesis protocols; (2) preparation of oligonucleotides having 3' terminal puromycin; (3) the linkage of the oligonucleotides to mRNA molecules; and (4) the in vitro translation of the mRNA-puromycin to create the protein-nucleic acid fusions. Thus, Exhibit B shows that as of February 10, 1994, we were able to provide a specific description of a procedure for making and using the claimed protein-nucleic acid fusions, as well as a clear description of the

structural components of the fusion molecules. Exhibit B also indicates that steps (1)-(3) had already been accomplished by February 10, 1994. This fact is confirmed by exhibits described below.

5. A fellowship application that I submitted to the National Institutes of Health ("NIH") for the National Research Service Award ("NRSA") further confirms our early conception of the claimed subject matter. The grant application was based on a research plan, research proposal, and preliminary data that we had already developed before December 1994. The NRSA application is attached hereto as Exhibit C.[1] This NRSA application was prepared with input from Dr. Szostak and was approved by him on November 17, 1994. The NRSA application was read and approved by Dr. Howard M. Goodman as the Department Head on November 29, 1994, and by the sponsoring institution on December 2, 1994, as shown on (Form Page 9) page 26 of the NRSA application. The NRSA application together with the face page signed by me on December 1, 1994 was received by the NIH on December 5, 1994 as shown by the official stamp/sticker on top of the face page of the NRSA application.

6. The NRSA application includes four figures that show our research design for preparing and using the protein-nucleic acid chimeras (pages 17-19). Figure 1 of the NRSA application is identical to Figs. 1A-1C of the present patent application and shows schematic representations of steps involved in the production of protein-RNA fusions. Figure 2 of the NRSA application is identical to Fig. 4 of the present patent application, which is a schematic representation of a preferred method for the preparation of protected CPG-linked puromycin. This is the form of puromycin that is suitable for use in the chemical synthesis of DNA or RNA. Figure 4 of the NRSA application is identical to Fig. 2 of the present patent application, which illustrates the use of protein-nucleic acid fusions in a protocol for selecting proteins having desirable properties. Therefore, the NRSA application provides further evidence that as of December 1, 1994, we had conceived of the claimed polypeptide-nucleic acid fusions, as well as uses for the claimed fusions

[1] The NRSA Application in Exhibit C was obtained by Landon Stark Cantwell & Paxton directly from the NIH under the Freedom of Information Act.

(e.g., generating libraries of diverse polypeptides and selecting those having desirable properties, along with the encoding nucleic acid) and detailed experimental plans for producing such fusions.

REDUCTION TO PRACTICE

7. The NRSA application provides evidence regarding reduction to practice for many of the intermediate steps in the preparation of the claimed protein-nucleic acid fusions. On page 11 of the NRSA application, in the "Progress to Date" section, I outlined my progress between May 1993 and November 1994 as a postdoctoral fellow in the Szostak laboratory. This section establishes that during this period of time I succeeded in synthesizing CPG-puromycin, synthesizing and testing puromycin-tethered oligonucleotides, and synthesizing mRNA-puromycin conjugates for in vitro translation to generate RNA-protein fusion molecules. This corresponds to the reduction to practice of steps (1)-(3) discussed in paragraph 4, above.

8. To further substantiate the reduction to practice of the first three intermediate steps, and to establish complete reduction to practice of the invention as a whole, I have attached hereto a copy of certain pages from my lab notebooks for experiments conducted and results obtained before October 17, 1996 (Exhibits D-K). I had kept my notes in ring binder notebooks. The notebooks, in their original condition, were labeled at each page with a sequential bates number starting with "P 00001" (of the first notebook entitled "Postdoc Proposal Notebook"), and notebooks pages were then scanned and imaged as pdf files and saved on CD-ROMs. The pages attached hereto are printed out from the pdf files in the CD-ROMs.

9. Notebook pages P 00237-00253 record my successful synthesis of CPG-Puromycin in December, 1993. See Exhibit D. CPG-Puromycin is a protected form of puromycin linked to a solid substrate that may be used in an automated DNA synthesizer for the production of 3'-puromycin tethered oligonucleotides.

10. P 00237 shows the beginning of a procedure for synthesizing CPG-Puromycin, which is a form of puromycin linked to a solid (controlled pore glass, "CPG") substrate that may be used in an automated DNA synthesizer for the production of 3'-puromycin tethered oligonucleotides. P00237

records the procedure I used to prepare the free base form of puromycin from a puromycin-HCl stock obtained from Sigma Co. I used spectrophotometric measurements to assess the concentration of puromycin in my preparation.

11. P 00238 records the preparation of "Trif Puromycin". "Trif" is a shorthand notation I used for the protecting group trifluoroacetic anhydride, $(CF_3CO)_2$. "Trif Puromycin" is puromycin in which the free amino group has been reacted with $(CF_3CO)_2$ to create a protected amino group that is inert in subsequent reactions. I reacted free base puromycin (noted as "MW = 472") with dry CH_3CN, dry pyridine, Trif anhydride and TEA (triethylamine). I monitored the reaction by thin layer chromatography ("TLC"), and, because TLC plates are not durable, I sketched the results in my notebook. By TLC, I was able to visualize a product that appeared to correspond to Trif Puromycin. P00239 shows spectrophotometric characterization of the reaction products. Trif Puromycin has a shifted absorbance spectrum in MeOH/$CHCl_3$ relative to unmodified puromycin, and I was able to determine that the reaction had proceeded to completion, converting approximately 100% the free puromycin to Trif puromycin.

12. P 00240 is dated December 2, 1993. This page records the preparation of dry Trif Puromycin for storage. Although the previous pages P 00238 and P 00239 are not dated, it is apparent that I could not have prepared Trif Puromycin for storage without first synthesizing it. Therefore I can state with confidence that the Trif Puromycin synthesis was conducted prior to December 2, 1993.

13. P 00241 records the beginning of my synthesis of "Trityl Trif Pur", a notation I used for puromycin in which the free amino group has been protected by reaction with trifluoroacetic anhydride and the 5' hydroxyl group has been protected by reaction with 4,4'-Dimethoxytrityl chloride (also named 4,4'-Dimethoxytriphenylmethyl chloride; DMT-Cl; and simply "Trityl"). I reacted previously prepared Trif-Pur with 4-Dimethylaminopyridine ("DMAP"), DMT-Cl, TEA and pyridine. By one- and two-dimensional TLC (P00241, 00242) I observed the production of partially and completely tritylated forms of Trif-Puromycin. P 00243-00246 record a large scale preparation of Trityl-Trif-Pur, showing a yield of 72% (P 00246).

14. P 00247-00251 record my procedure for succinylating Trityl Trif Pur (also referred to as "DMT-Trif Pur"). Succinylation prepares the Trityl Trif Pur for cross-linking to a solid substrate. Among these pages, P 00248-00249, showing a small scale pilot experiment, are dated December 13, 1995 while P 00250-00251, recording the large scale preparation, are dated December 13 and December 14, 1993. It was my standard practice to perform a small experiment prior to the large scale reaction, and therefore I conclude that P 00248 is erroneously dated "1995" and should read instead "1993". Regardless, it is clear from the dates given that I performed a large scale succinylation of Trityl Trif Pur on December 13 and 14 of 1993. Although the preceding pages P 00241-00246, discussed above, are not dated, I could not have performed the succinylation of Trityl Trif Pur without first preparing the Trityl Trif Pur. Therefore, I can state that I had prepared Trityl Trif Pur, as shown in pages P 00241-00246, prior to December 13, 1993.

15. P 00252-00253 record the coupling of succinate-DMT-Trif-Pur to a solid support. The "nucleoside" (succinate-DMT-Trif-Pur) was reacted with dioxane, paranitrophenol, pyridine and dicyclohexylcarbodiimide ("DCC"). The reaction product was reacted with a solid substrate, controlled pore glass (CPG) (P 00253). I obtained a 22% yield. CPG is commonly used as the solid substrate for solid phase automated DNA and RNA synthesis. CPG-Puromycin is suitable for use in the synthesis of 3'-puromycin-linked oligonucleotides.

16. On April 3, 1994, I presented my progress in preparing RNA-Puromycin conjugates to the members of the Szostak laboratory (P 00466-00486, Exhibit E). This presentation summarizes my difficulties in preparing a puromycin-nucleic acid conjugate through liquid phase synthetic chemistry (P 00467) and my successful strategy for preparing CPG-Puromycin for use in solid phase nucleic acid synthesis (P 00468), as described above. Taken together, Exhibits D and E establish that, prior to April 3, 1994, I had reduced to practice the synthesis of CPG-Puromycin.

17. The April 3, 1994 presentation also contains notebook records of my reduction to practice of an oligonucleotide-puromycin conjugate. P 00469 is an undated NMR tracing showing, at bottom, the product of a successful synthesis of a cytosine-puromycin dinucleotide ("pCpP"). P 00470 shows a similar scan dated January 13, 1994. These conjugates were made manually using the

CPG-Puromycin as the starting material. The deprotection scheme resulted in the puromycin conjugate in a mixture with TBAF (tetrabutyl ammonium fluoride) in THF (tetrahydrofuran).. I was able to separate the puromycin-conjugated nucleic acids from the TBAF by high performance liquid chromatography (HPLC) (P 00471-00478). The chromatography trace on P 00474 shows a sloping line, representing increasing salt concentration in an anion exchange column, and a single peak eluting, representing the elution of pCpCpP (5' phosporylated cytosine-cytosine-puromycin). The elution profile on P 00476 shows a more complicated elution profile for the smaller, less charged pCpP. The tracing on P 00478 is dated February 8, 1994, and shows the elution of pdCpP (deoxycytosine-puromycin) in a clean, isolated peak. The chromatograms are marked to indicate fractions that I took. Therefore, I had successfully produced a cytosine-puromycin conjugate by January 13, 1994 and by February 8, 1994, I was able to synthesize short oligonucleotide-puromycin conjugates and purify these conjugates by anion exchange chromatography.

18. The April 3, 1994 presentation also contains illustrations and notebook records of my early attempts at preparing mRNA-puromycin conjugates. P 00479-00480 illustrate four possible template designs for mRNA-puromycin conjugates. P 00481 illustrates two general strategies for attaching a puromycin ("P") to an mRNA using either DNA ligase or RNA ligase. P 00482-00486 illustrates some of my preliminary experiments addressing both strategies. P 00482 illustrates that small quantities of radioactively labeled 25.137 (a puromycin oligonucleotide constructed by automated synthesis) can be attached to two different mRNAs via DNA ligase. P 00484 illustrates that radioactively labeled pCpP can be attached to an RNA using RNA ligase.. Thus, by April 3, 1994, I had successfully prepared a CPG-puromycin, synthesized oligonucleotide-puromycin conjugates and attached such conjugates to RNA. This timeline corresponds with the timeline presented in Exhibits B and C.

19. The materials in Exhibits F-K demonstrate that, prior to October 17, 1996, I had successfully conducted in vitro translation of the mRNA-puromycin conjugates to produce the protein-nucleic acid fusions. Before I submitted the NRSA application in December 1994, I had obtained mRNA-puromycin conjugate and I had been working on in vitro translation of the conjugate to obtain the RNA-protein fusion molecules. See Exhibit F (notebook pages P 00580- P 00581; P 00585- P

00594;P 00753-P 00767). P 00580-P 00594 record an experiment conducted in June 1994, and the results indicate that I had obtained the mRNA-puromycin conjugate and was able to in vitro translate the conjugate (also referenced to as "chimera" in my notebook) to generate the desired fusion product. P 00753-P 00767 record an experiment conducted in October 1994 on in vitro translation of an mRNA-puromycin conjugate (43.33), termed "43-P" in the present patent application using *E. coli*-based in vitro translation system. Figure 6A in the present patent application is the mirror image of the autoradiograph shown on P 00759. In particular, the results show the incorporation of ^{35}S methionine in the mRNA-puromycin conjugate template after in vitro translation as a function of Mg^{2+} concentration. On June 7, 1995, I also conducted an experiment on in vitro translation of an mRNA-puromycin conjugate 43.33 using reticulocyte lysate. See Exhibit G (notebook pages P 01004-P 01007). As shown on P 01006, bottom half of the page, the mRNA-puromycin conjugate 43.33 was successfully translated as indicated by the incorporation of ^{35}S-Met into the translation template, and the autoradiography was obtained after overnight exposure on June 8, 1995. On June 8, 1995, I repeated the in vitro translation experiment. See Exhibit G (notebook pages P 01008-P 01009). As shown on P 01009, the autoradiography dated June 8-June 9, 1995 (overnight exposure), the mRNA-puromycin conjugated was also successfully translated in vitro. Fig. 6H of the present patent application, in fact, is from (and is the mirror image of) the autoradiograph obtained on June 9, 1995, as shown on P 01009. On October 31, 1995, I also conducted an experiment to generate a mRNA-puromycin conjugate linked to a polypeptide (notebook pages P 001165- P 001167) using both the reticulocyte lysate and wheat germ translation extracts. Fig. 8 of the present patent application, in fact, is from the autoradiograph dated November 3, 1995. Therefore, as of October 31, 1995, I had translated an mRNA-puromycin conjugate in vitro to generate a mRNA-polypeptide fusion.

20. On November 1, 1995, I conducted immunoprecipitation experiments using an RNA-protein fusion molecule preparation, of which the protein portion included a c-myc epitope and is encoded by the RNA portion of the fusion molecule. See Exhibit H (notebook pages P 01181-P 01183). On P 01181, I showed the experimental design (with a schematic representation of the components involved in the immunoprecipitation) and protocol. On the top half of P 01182, an autoradigraph

obtained after three-day exposure, a band was present in the lane marked as "LP77" with antibody, showing that the RNA-protein fusion molecule indeed had the c-myc epitope and that the protein portion of the fusion molecule was bound (and thus immunoprecipitated) by the antibody. A four-day exposure, as shown on P 01183, turned up a light band in the lane marked as "LP155" with antibody (the band was at a higher molecule weight and about the same position as the higher molecular weight band in the positive control lane of "LP155"), indicating the presence of the peptide epitope in the LP155 RNA-protein fusion molecule and the binding of the peptide epitope by the antibody. "LP77" and "LP155" as used in the notebooks are identical to the terms "LP77" and "LP154,"[2] respectively, as used in the present patent application. See Figs. 7A and 7B of the present patent application for the schematic representations of the structures and peptide sequences for "LP77" and "LP154," respectively. Therefore, as of November 1995, I had translated a polypeptide from an mRNA-puromycin conjugate and I had confirmed by antibody binding that the protein produced was in fact the protein encoded by the mRNA-puromycin conjugate.

21. On June 26, 1996, I submitted an NRSA Continuation Application, which requested continued funding from September 1, 1996 and February 28, 1997. The Continuation Application may be found as the last four pages of the NRSA application in Exhibit C. The Continuation Application was approved by Dr. Szostak, Dr. Goodman, and the sponsoring institution in June 1996. The Continuation Application was accepted by the NIH, and the fellowship award was issued on August 31, 1996. In this Continuation Application, I reported to the NIH the research that had been completed (page 2 of the Continuation Application), which had followed the timetable set forth in the original research proposal. I reported the difficulties that I had experienced and how I had overcome them to successfully produce RNA-protein fusion molecules. In addition, I described experiments to optimize translation conditions so as to maximize the amount of fusion generated. This submission to the NIH corresponds to and confirms the timeline established in my laboratory notebooks, illustrating the reduction to practice of the claimed protein-nucleic acid chimeras earlier than June 26, 1996.

[2] The discrepancy of terms "LP154" and "LP155" was due to an initial error in calculating the chain length.

22. In August 1996, I prepared two presentations. Exhibits I (August 14, 1996 presentation, notebook pages P 01693-P 01723) and J (August 26, 1996 presentation, notebook pages P 01727-P01737). I prepared slides for these two presentations, of which several also became figures included in the present patent application. For example, Figs. 6B and 6D of the present patent application are from two figures on P 01700. Figs. 6A and 6C of the present patent application are from two figures on P 01701. Fig. 9 of the present patent application is from the figure initially obtained on May 9, 1996, as shown on P 01705 as well as P 01731, which demonstrates protease sensitivity of the RNA-protein fusion synthesized from LP154. These presentations describe the entire scheme for preparing the protein-nucleic acids of the claimed invention and provide data collected over the previous years summarizing the reduction to practice.

23. In September 1996, I conducted experiments on different ways to isolate and purify the RNA-protein fusion molecules. See Exhibit K (notebook pages P 01832-P 01837). As shown on P 01832, I outlined the experimental design and protocol on September 17, 1996. The isolated and radio-labeled samples were measured for their radioactivity with scintillation counter.[3] I ran gel electrophoresis on the isolated samples, and autoradiographs were taken after exposure for different time lengths. For example, on P 01837, after 36-hour exposure, on September 20, 1996, the autoradiograph showed purified RNA-protein fusions in different lanes prepared by different purification methods.

24. To conclude, Exhibits B and C establish conception of the claimed protein-nucleic acid fusions by February 10, 1994 and December 1, 1994, with latter being an official grant application submission to the NIH that was subsequently funded. Exhibits B and C also describe intended uses for the protein-nucleic acid chimeras (e.g., generating libraries of diverse polypeptides and selecting those having desirable properties, along with the encoding nucleic acid) and present a road map of the specific experiments by which the synthesis of the claimed compositions would eventually be accomplished. Exhibits D-K establish the reduction to practice of the claimed protein-nucleic acid chimeras well before October 17, 1996 and provide evidence of the research that I conducted to

[3] The scintillation counter results were shown on P 01833 and P 01834. However, the printer attached to the counter showed a 1984 date, clearly incorrect.

achieve: (1) preparation of puromycin in a form that is suitable for use with standard DNA/RNA synthesis protocols; (2) preparation of oligonucleotides having 3' terminal puromycin; (3) the linkage of the oligonucleotides to mRNA molecules; and (4) the in vitro translation of the mRNA-puromycin to create the protein-nucleic acid fusions.

The undersigned declares further that all statements made herein of his own knowledge are true and all statements made on information and beliefs are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issuing thereon.

Richard W. Roberts, Ph.D.

Date: 10/6/04